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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Hansell	STEDMAN	Philadelphia, PA
Leonard	SU	Philadelphia, PA
Marilyn	MITCHELL	Philadelphia, PA

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

22264 U.S. PTO 60/538877 012304

TITLE OF THE INVENTION (500 characters max)

AAV MICROUTROPHIN AND METHODS OF USE THEREOF

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number — Type Customer Number here — Place Customer Number Bar Code Label here

OR

■ Firm *or* Individual Name	Lisa Burgin Conte, Esquire				
Address	Dilworth Paxson LLP				
Address	3200 Mellon Bank Center, 1735 Market Street				
City	Philadelphia	State	Pennsylvania	ZIP	19103
Country	US	Telephone	215.575.7356	Fax	215.575.7200

ENCLOSED APPLICATION PARTS (*check all that apply*)

■ Specification *Number of Pages* 5 — ☐ CD(s), Number

■ Drawing(s) *Number of Sheets* 0 — ☐ Other (specify):

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

■ Applicant claims small entity status. See 37 CFR 1.27.

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☑ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account No. 50-0979.

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

■ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Lisa Burgin Conte

Lisa Burgin Conte, Reg. No. 52,470

Date: January 23, 2004

Attorney Docket No. Q3355

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

EV222867963US

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AAV MICROUTROPHIN AND METHODS OF USE THEREOF

Description of the Technology:

This document discloses the construction and intended use of a microutrophin coding sequence in the treatment of the most common X-linked lethal disease in man. The goal is to use this new construction in the context of recombinant AAV delivered to skeletal and ultimately cardiac muscle as outlined in previous technology disclosures.

Duchenne Muscular Dystrophy (DMD) is caused by a deficiency of the muscle cytoskeletal protein known as dystrophin(Hoffman, Brown et al. 1987; Hoffman, Fischbeck et al. 1988). Dystrophin is a member of the spectrin superfamily of proteins and as such is distantly related to spectrin and alpha-actinin(Koenig, Monaco et al. 1988). Dystrophin is most closely related to the protein utrophin(Tinsley, Blake et al. 1992). The genes for these two proteins have nearly identical intron/exon structures, and the proteins are 50+% homologous at the amino acid level. Dystrophin is expressed throughout the entire length of the skeletal muscle fiber while utrophin is normally expressed only at the neuromuscular junction. Most cases of DMD result from sporadic deletions of the X chromosomal dystrophin gene(Koenig, Beggs et al. 1989). The destruction of the dystrophin open reading frame by these mutations suggests that therapies that genetically reconstitute dystrophin expression will elicit a cellular immune response against the fibers in which the protein is synthesized.

In the years following the initial discovery of utrophin, the technologies for targeted gene ablation in mice facilitated a formal genetic analysis of gene complementation. In the transgenic mouse in which the expression of utrophin is dictated by a muscle-specific promoter, utrophin can complement the physiological role of dystrophin(Tinsley, Potter et al. 1996; Tinsley, Deconinck et al. 1998). This has prompted a multi-million dollar reasearch effort to find pharmacological means of upregulating the expression of utrophin in the muscle of patients with DMD(Burton, Tinsley et al. 1999; Perkins, Burton et al. 2001).

Our strategy is different: somatic transfer of a micro-utrophin encoding DNA sequence under the control of a muscle-specific promoter(Stedman 2001). Recently published studies from several groups have demonstrated the utility of AAV-sized microdystrophin cassettes for reversing the pathology of dystrophin deficiency in

mice(Wang, Li et al. 2000; Harper, Hauser et al. 2002). Building on this advance, we have constructed a microutrophin cassette for use in probing both the functional restoration of dystrophin and the immune response. Our preferred animal model for these studies is the German Short Haired Pointer dog, because of its complete deletion of the dystrophin coding sequence(Schatzberg, Olby et al. 1999). All other "dystrophin-deficient" animal models described to date derive from point mutations, with the end result that the immune systems in these animals are predicted to develop tolerance to the peptide encoded by the remainder of the dystrophin open reading frame(Schatzberg, Anderson et al. 1998; Lu, Morris et al. 2000). In the GSHP dog model we will be able to study in detail the immune response to recombinant canine dystrophin and utrophin, when these proteins are produced from somatically delivered AAV vectors. On completion of these studies we will have answered essential questions about the relative safety and efficacy of the two methods for treating DMD by somatic gene transfer.

Sequence 1
Microutrophin Nucleotide Sequence

ATCGATCCACCATGGCCAAGTATGGAGAACATGAAGCCAGTCCTGATAATGGGCAGAACGAATTCAGTGACATCATTAA
GTCCAGATCTGATGAACACAATGACGTGCAGAAGAAAACCTTTACCAAATGGATCAATGCGCGATTTTCAAAGAGTGGA
AAACCACCCATCAATGATATGTTCACAGACCTCAAAGATGGAAGGAAGCTCCTGGATCTTCTGGAAGGCCTCACAGGAA
CATCACTGCCAAAGGAACGTGGTTCCACAAGGGTACATGCTTTAAATAATGTCAACAGAGTGCTGCAGGTTTTGCATCA
GAATAATGTGGATTTAGTGAATATAGGAGGAACTGACATTGTAGATGGAAATCACAAACTGACTTTGGGATTACTTTGG
AGCATCATTTTGCACTGGCAGGTAAAAGATGTCATGAAAGATGTCATGTCAGACCTGCAGCAGACAAACAGTGAGAAGA
TCCTACTGAGCTGGGTGCGCCAGTCTACTAGGCCGTACAGCCAGGTCAACGTCCTCAACTTCACCACCAGCTGGACAGA
TGGACTGGCCTTTAATGCTGTGCTGCACCGACATAAACCTGATCTCTTCAGCTGGGATAGAGTTGTCAAAATGTCCCCA
ATTGAGAGACTTGAACATGCCTTCAGCAAAGCTCAAACTTATTTGGGAATTGAAAAGCTGTTAGATCCTGAAGATGTTG
CCGTTCAACTTCCTGACAAGAAATCCATAATTATGTATTTAACATCTTTGTTTGAGGTGCTTCCTCAGCAAGTCACTCT
AGATGCCATCCGTGAAGTAGAGACACTCCCAAGGAAATATAAGAAAGAATGTGAAGAAGGAGAGATTAGTATACAGAGC
TCAGCGCCAGAGGAGGAGCATGAGTGTCCCGGAGCTGAAACCCCCAGCACTGTCACTGAAGTTGACACGGATCTGGACA
GCTATCAGATAGCACTGGAGGAAGTGCTGACCTGGTTGCTTTCTGCCGAGGACACTTTCCAGGAGCAGGATGACATTTC
TGATGATGTAGAAGAAGTCAAAGAGCAGTTTACTACCCATGAAGCTTTTATGATGGAGCTGACAGCGCACCAGAGCAGT
GTGGGCAGTGTCCTGCAGGCAGGAAACCAGCTGATAACGCAAGGAACTCTGTCAGATGAGGAGGAATTTGAAATTCAGG
AACAAATGACCCTGCTAAATGCTAGATGGGAGGCACTCAGGGTGGATAGTATGAACAGACAGTCCCGGCTGCATGATGT
GTTGATGGAACTACAAAAGAAGCAGTTGCAACAGCTCTCTGCCTGGTTAACACTCACAGAAGAACGCATTCAGAAGATG
GAAACCTGCCCCCTGGATGATGATTTAAAATCCCTACAAAAGCTACTAGAAGATCATAAACGTTTGCAAAATGATCTTG
AGGCGGAACAGGTGAAGGTAAATTCACTAACACACATGGTGGTGATTGTTGATGAAAACAGTGGTGAGAGTGCCACTGC
TGTTCTGGAAGATCAGTTACAGAAACTTGGTGAACGCTGGACAGCAGTGTGCCGTTGGACAGAGGAACGTTGGAGTAGG
CTACAAGAAATTAATATATTGTGGCAGGAATTATTAGAAGAACAGTGCTTGTTGAAAGCTTGGCTAACTGAAAAAGAAG
AGGCCTTAAATAAAGTCCAGACGAGCAACTTCAAAGACCAAAAGGAACTAAGTGTCAGCATCCGACGATTGGCTATTTT
GAAGGAAGACATGGAAATGAAACGTCAGGCATTGGATCAGCTAAGTGAGATTGGCCAGGATGTGGGTCAATTAGTTGAT
AATCCCAAGGCATCTAAGAAGATCAACAGTGACTCAGAGGAACTAACTCAGAGATGGGATTCTTTGGTTCAGAGACTAG
AAGATTCCTCTAACCAGGTGACTCAGGCTGTGGCAAAGCTGGGGATGTCCCAAATTCCTCAGAAAGATCTTCTGGAGAC
TGTTCGCATAAGAGAACAAGTAACTACAAAAAGGTCTAAGCAAGAACTGCCTCCTCCTCCTCCCCCAAAGAAGAGACAG
ATTCCTGTCGACCTGGAGAAGCTCAGAGACCTGCAGGGAGCCATGGATGACCTGGATGTTGACATGAAGGAGGCGGAGG
CTGTGAGGAATGGCTGGAAGCCTGTGGGAGACTTACTTATCGACTCACTGCAGGATCACATTGAAAAAACCATGGCATT
TAGAGAAGAAATTGCACCAATCAACCTAAAAGTTAAAACAGTGAATGATTTATCCAGTCAGCTGTCTCCACTTGACCTG
CATCCATCTCTAAAGATGTCTCGCCAGCTAGATGACCTTAATATGCGATGGAAACTTCTGCAGGTTTCTGTGGATGATC
GCCTTAAACAGCTTCAGGAAGCCCATAGAGATTTTGGGCCATCCTCTCAGCATTTTCTTTCTACTTCAGTCCAGCTGCC
ATGGCAAAGATCCATTTCACATAATAAAGTGCCCTATTACATCAACCATCAAACACAGACAACTTGTTGGGACCGTCCT
AAAATGACTGAACTCTTTCAATCTCTTGCTGACCTGAATAATGTACGTTTCTCTGCCTACCGTACAGCCATCAAAATCC
GAAGACTACAAAAAGCACTGTGTTTGGATCTCTTAGAGTTGAATACAACAAATGAAGTTTTCAAGCAGCACAAACTGAA
CCAAAATGATCAGCTTCTTAGCGTTCCAGATGTCATCAACTGTCTGACAACAACTTATGATGGTCTTGAACAAATGCAT
AAGGATCTGGTCAACGTTCCACTCTGTGTGGATATGTGTCTCAACTGGTTGCTCAATGTGTATGACACGGGTCGAACTG
GAAAAATAAGAGTGCAGAGTCTGAAGATTGGATTGATGTCTCTCTCCAAAGGTCTCTTAGAAGAAAAATACAGATATCT
CTTTAAGGAGGTGGCAGGTCCGACAGAAATGTGTGACCAGAGGCAGCTTGGCCTGTTACTTCATGATGCCATCCAGATC
CCTCGGCAGCTGGGGGAAGTAGCAGCTTTTGGGGGCAGTAATATTGAACCCAGTGTTCGCAGCTGCTTCCAACAGAATA
ACAATAAGCCAGAGATAAGCGTAAAAGATTTTATAGATTGGATGCGTCTGGAACCACAGTCCATGGTTTGGCTGCCAGT
TTTACACCGAGTGGCTGCAGCTGAGACTGCAAAGCATCAAGCTAAATGCAACATCTGTAAAGAATGTCCAATAGTTGGG
TTCAGGTATAGAAGCCTAAAGCATTTTAACTATGATGTCTGCCAGAGTTGCTTTTTTTCGGGTCGAACGGCAAAAGGTC
ACAAATTACATTACCCAATGGTGGAATATTGTATACCTACAACATCTGGGGAAGATGTACGAGACTTCACAAAGGTGCT
GAAGAATAAGTTCAGATCAAAGAAATACTTTGCCAAACATCCTCGGCTTGGCTACCTGCCTGTCCAGACAGTACTTGAA
GGTGACAACTTAGAGACTTGAAAAACTCGAG

Sequence 2
Microutrophin Peptide Sequence

MAKYGEHEASPDNGQNEFSDIIKSRSDEHNDVQKKTFTKWINARFSKSGKPPINDMFTDL
KDGRKLLDLLEGLTGTSLPKERGSTRVHALNNVNRVLQVLHQNNVDLVNIGGTDIVDGNH
KLTLGLLWSIILHWQVKDVMKDVMSDLQQTNSEKILLSWVRQSTRPYSQVNVLNFTTSWT
DGLAFNAVLHRHKPDLFSWDRVVKMSPIERLEHAFSKAQTYLGIEKLLDPEDVAVQLPDK
KSIIMYLTSLFEVLPQQVTLDAIREVETLPRKYKKECEEGEISIQSSAPEEEHECPGAET
PSTVTEVDTDLDSYQIALEEVLTWLLSAEDTFQEQDDISDDVEEVKEQFTTHEAFMMELT
AHQSSVGSVLQAGNQLITQGTLSDEEEFEIQEQMTLLNARWEALRVDSMNRQSRLHDVLM
ELQKKQLQQLSAWLTLTEERIQKMETCPLDDDLKSLQKLLEDHKRLQNDLEAEQVKVNSL
THMVVIVDENSGESATAVLEDQLQKLGERWTAVCRWTEERWSRLQEINILWQELLEEQCL
LKAWLTEKEEALNKVQTSNFKDQKELSVSIRRLAILKEDMEMKRQALDQLSEIGQDVGQL
VDNPKASKKINSDSEELTQRWDSLVQRLEDSSNQVTQAVAKLGMSQIPQKDLLETVRIRE
QVTTKRSKQELPPPPPPKKRQIPVDLEKLRDLQGAMDDLDVDMKEAEAVRNGWKPVGDLL
IDSLQDHIEKTMAFREEIAPINLKVKTVNDLSSQLSPLDLHPSLKMSRQLDDLNMRWKLL
QVSVDDRLKQLQEAHRDFGPSSQHFLSTSVQLPWQRSISHNKVPYYINHQTQTTCWDRPK
MTELFQSLADLNNVRFSAYRTAIKIRRLQKALCLDLLELNTTNEVFKQHKLNQNDQLLSV
PDVINCLTTTYDGLEQMHKDLVNVPLCVDMCLNWLLNVYDTGRTGKIRVQSLKIGLMSLS
KGLLEEKYRYLFKEVAGPTEMCDQRQLGLLLHDAIQIPRQLGEVAAFGGSNIEPSVRSCF
QQNNNKPEISVKDFIDWMRLEPQSMVWLPVLHRVAAAETAKHQAKCNICKECPIVGFRYR
SLKHFNYDVCQSCFFSGRTAKGHKLHYPMVEYCIPTTSGEDVRDFTKVLKNKFRSKKYFA
KHPRLGYLPVQTVLEGDNLET

We Claim:

1. A microutrophin cassette for treatment of Duchenne Muscular Dystrophy (DMD) by somatic gene transfer.

2. A method of using the microutrophin cassette of claim 1 for restoration of dystrophin.

3. A method of using the microutrophin cassette of claim 1 to generate an immune response.

4. A method of treating dystrophin deficiency by somatic gene transfer.

5. The nucleotide sequence embodied in sequence 1 that encodes a microutrophin molecule, wherein the microutrophin molecule is homologous to the human dystrophin homolog utrophin.

6. A microutrophin molecule embodied in the polypeptide sequence of sequence 2, wherein the microutrophin molecule is homologous to the human dystrophin protein homolog utrophin.

7. A method of treatment using the nucleotide sequence of claim 5 wherein the nucleotide sequence is delivered to human cells by one or more gene vectors from the group comprising adenovirus, adeno associated virus, lentivirus and plasmids.

8. A method of using the sequence of claim 5 in gene therapy applications to treat muscle disorders.

9. A method of using the sequence of claim 5 in gene therapy applications to treat muscular dystrophy.

10. A method of using the sequence of claim 5 in gene therapy applications to treat Duchenne Muscular Dystrophy.

11. A method of using the microutrophin molecule of claim 6 to treat muscle disorders.

12. A method of using the microutrophin molecule of claim 6 to treat muscular dystrophy.

13. A method of using the microutrophin molecule of claim 6 to treat Duchenne Muscular Dystrophy.

14. A nucleotide sequence that is at least 50% homologous to the nucleotide sequence of claim 5.

15. A polypeptide sequence that is at least 50% homologous to the polypeptide sequence of claim 6.